

RESPONSES OF THE SWIMBLADDER OF THE GUPPY,  
*LEBISTES RETICULATUS*, TO SUDDEN  
PRESSURE DECREASES

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Most fishes maintain a density equal to that of the surrounding water. This is done through a very accurate control of the volume of their swimbladders by appropriate gaseous exchange between the swimbladder and the blood. As the fish passes into deeper water the increased hydrostatic pressure compresses the bladder gases to the point that the fishes are no longer buoyed up in the water. Consequently, in order for the fishes to adapt themselves to the new pressure, they must put more gases into the bladder. Conversely, as the fishes rise in water the decreasing hydrostatic pressure renders the gases in the bladder too buoyant and provision is made for the release of the proper amount. Many experiments have been performed in an attempt to determine the mechanism of control of the swimbladder. The great majority of these experiments have involved analysis of gases found in the swimbladder in different states of adaptation and under controlled experimental conditions. Although much valuable information has been obtained, our picture of the mechanism is by no means complete.

Von Ledeber (1937) summarizes our present knowledge in this field and reviews briefly all the current theories to explain the secretion of gases into the swimbladder against the diffusion gradient and their removal. The variety of explanations indicates the need of more research before a definitive theory can be devised.

The experiments to be described in this report were carried out upon the guppy, *Lebistes reticulatus*, a physoclistous fish. They demonstrate the responses of the swimbladder to decreased hydrostatic pressure and to situations in which the normal diffusion gradient, favoring passage of gases out of the bladder, is experimentally reversed and varied in steepness. Some interesting information has been obtained upon the mechanism of gaseous exchange between the environment and swimbladder.

The apparatus consisted of a two and a half gallon carboy. Through the stopper was projected a small bore glass tube which passed into a flask serving as an air cushion. To this flask was attached a mercury manometer and tubes which led to a vacuum pump on the one hand and

a compressor on the other. Both of the latter tubes were equipped with stopcocks. The whole system was arranged so that the pressure within the system could be rapidly changed but always kept under complete control. The experimental animal was placed in the carboy completely filled with water which rose three or four inches into the small bore glass tube of the stopper. In a few experiments where the gases present in the water were to be equilibrated to each new pressure by shaking, a liter bottle half full of water was substituted for the carboy.

With this equipment it was possible either to alter the pressure upon the fish without appreciably altering the gaseous concentration of the medium,<sup>1</sup> or to subject the animal to atmospheric pressure in water which had been equilibrated with air under heightened or lowered pressure.

With this same apparatus it was a simple matter to measure any changes in the total amount of gases in the bladder by the application of Boyle's law. The method was merely a refinement of the technique used by Evans and Damant (1928). From time to time the exact pressure to which the guppy was adapted was measured. As the amount of gases in the bladder increased, proportionately more pressure had to be applied in order to return the fish to the same density as the water, and conversely, as the fish permitted gases to escape from the bladder, proportionate decrease in pressure was necessary to adjust artificially the fish density so that the fish would tend neither to rise nor sink. The validity of this method depends upon the assumption that the pressure of the bladder gases results solely from the external environment about the fish. Evans and Damant found this to be the case with the physoclistous fish they examined, the bladder gas not being held under pressure by any structure of the fish body.

Some initial experiments were performed with completely normal guppies, but it was found difficult to make accurate readings of the exact pressure to which the fish was adapted at any given moment. This difficulty was a result of the fish's being able to maintain itself quite stationary in the water through an appreciable range of pressures by very slight activity of the pectoral fins. Consequently, all experiments reported here were conducted with fishes whose pectoral fins were amputated. This last procedure permitted equilibrium readings of considerable accuracy in a few seconds of time. There has been no reason to suspect that this fin removal affects the swimbladder response in any manner.

Such a method as the one used here may be applied to any physoclistous fishes since in these fishes the change in gas quantity takes place

<sup>1</sup> When not otherwise specified in the following experiments, the gaseous content of the water was that resulting from equilibrium with air at room temperature and the atmospheric pressure (about 750 mm. Hg).

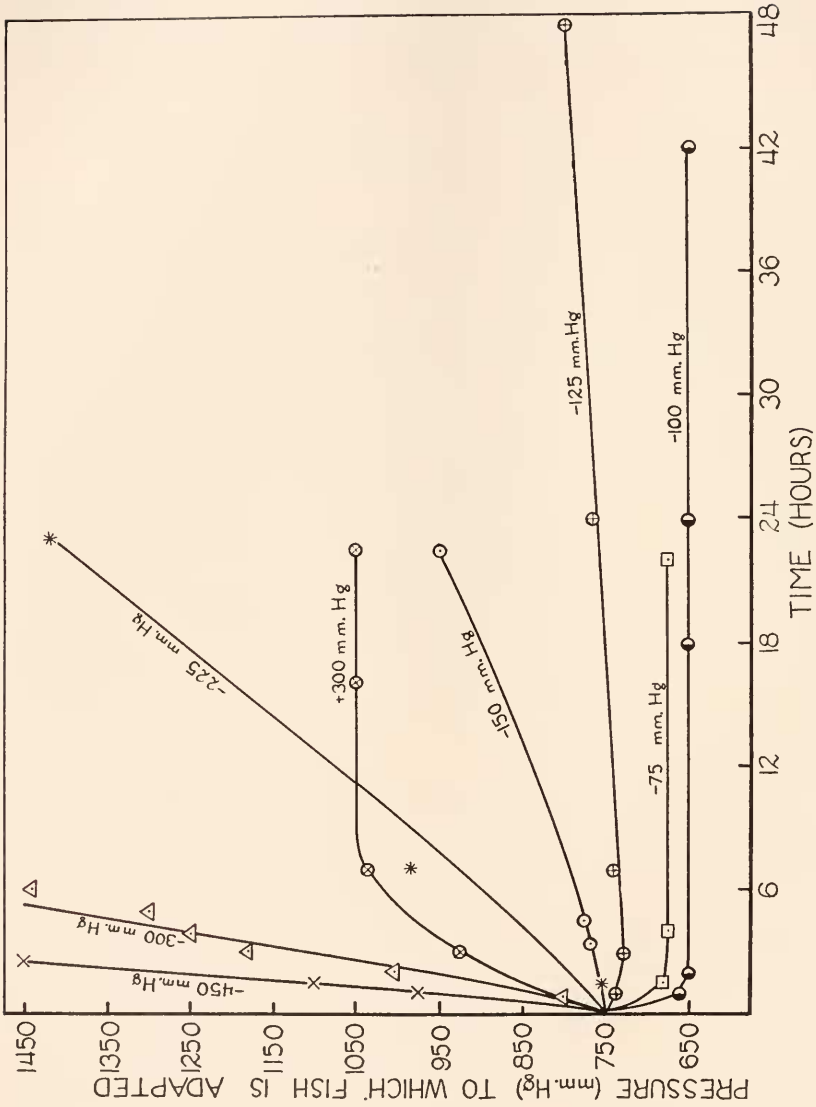


FIG. 1. Diagram showing the responses of the swimbladder to decreases in pressure of 75, 100, 125, 150, 225, 300, and 450 mm. Hg, and an increase of 300 mm. Hg.

only very slowly by secretion or diffusion of gases and hence the few seconds of sudden change of pressure for the purpose of determining the direction and extent of gas change cannot result in an abrupt change in gas content. Physostomous fishes such as the goldfish, on the other hand, almost instantly discharge gas bubbles by way of the mouth when the pressure is suddenly decreased.

#### EXPERIMENTAL

The first experimental series was designed to test the effect of changes in pressure upon the fish swimbladder response. Water was equilibrated with air at room temperature and at atmospheric pressure and placed in the carboy. A female guppy was permitted to adjust its density to that of the water in the new situation: a pressure of one atmosphere plus eighteen inches of water. This situation served as a starting point for each experiment. In as many separate experiments the pressure on the fishes was decreased by 75, 100, 125, 150, 225, 300, and 450 mm. Hg and increased by 300 mm. Hg. The responses of the fish to these changes are indicated in Fig. 1.

The fishes responded to increased pressure by decreasing their density gradually over the course of six or eight hours to the point of equaling that of the water. This density was maintained as long as the pressure was held constant. When the pressure was decreased by 75 or 100 mm. Hg, the fish increased its density to a value equal to the surrounding water.

However, as the pressure was decreased more, by 125, 150, 225, 300, and 450 mm. Hg, the fish no longer adaptively adjusted its density. Instead, its density decreased at a rate that was obviously a function of the amount of decrease in pressure. These results were confirmed by a second experiment in which only male guppies were used.

With pressure decreases of more than 100 mm. Hg the density of the fish continued to decrease for several days at a nearly constant rate, or until the swimbladder became so large that the wall of the visceral cavity was distended in a balloon-like fashion. When the pressure was decreased by 300 or more mm. Hg such a state was reached in less than twenty-four hours.

There thus appeared to be some level of pressure below which the fish was unable to adapt itself, and on the contrary, became more and more maladjusted. This pressure level appeared to be quite constant whether it was reached in one sudden pressure change, in two equal changes over several hours, or in three changes over a period of four days. Figures 2*A* and 2*B* illustrate results of this nature that were

obtained. Furthermore, the rate of decrease in fish density in response to a decreased pressure appeared to be independent of the rate at which the value was approached.

#### INTERPRETATION OF THE EXPERIMENTAL RESULTS

The cause of the abrupt reversal of fish response from density increase to decrease as the pressure was decreased by values greater than

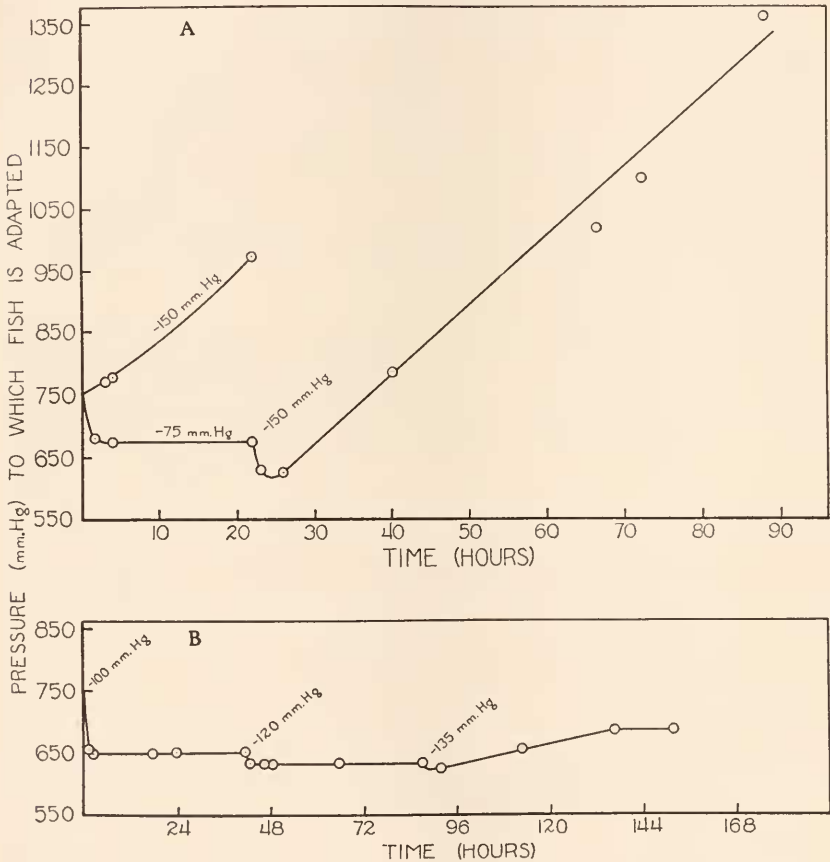


FIG. 2. (A) Graph showing the swimbladder responses to reduction in pressure when the reduction is carried out in a single step, in two steps over a period of about 20 hours, and (B) three steps over a period of about four days.

about 120 mm. Hg was the first question to be answered. A guppy adapted to a pressure increase of 300 mm. Hg, when suddenly returned to the original pressure, responded by increasing its density to a value

again equal to that of water. On the contrary, a guppy directly subjected to a similar percentage decrease in pressure responded in an opposite fashion by decreasing its density. Therefore, a physically induced size increase of the swimbladder and any activity or response of the fish directly correlated with such increase would not totally explain the situation. This eliminated any reflex associated with the eyes, fins, etc., as was indicated by Meesters and Nagel (1934) to be true over the normal pressure range.

A brief additional experiment was performed which immediately eliminated any induced change in amount of gases dissolved in the water as stimulating the bloating phenomenon. A guppy in an unstoppered flask of water was placed in a vacuum desiccator. The pressure on the fish was then decreased by 300 mm. Hg. The fish commenced to bloat. The water now had a dissolved air content found in conjunction with a decreased pressure which stimulated the bloating of the fish. The flask was quickly removed from the desiccator and paraffin oil poured upon the water surface to prevent any re-diffusion of gases into the water at the restored pressure. If decreased concentration of any gases in the water was responsible for the bloating phenomenon, such bloating would be expected to continue. The fish in this situation increased its density to a value equal again to that of water.

It was highly improbable that the fish was responding to pressure through the activity of any sense organ. The evidence indicated that if a postulated hydrostatic pressure sense organ was actually operating it displayed no signs of adaptation or fatigue (see especially Fig. 2*B*). A physical explanation thus appeared more plausible.

Two further and decisive experiments were performed which demonstrated the character of this pressure effect. A guppy was placed in a bottle only partially filled with water. The water was thoroughly shaken with air at each reduced pressure. The fish adjusted its density to agree with that of the water for all the pressure decreases used. In one experiment the pressure was decreased by 300 mm. Hg. Again, when water was thoroughly shaken with air under a pressure increase of 500 mm. Hg, the guppy bloated when placed in this after the pressure had been restored to the original value. The rate of bloating was of the same order of magnitude as seen in response to a pressure decrease of 300 mm. Hg.

A partial interpretation of the experimental results thus seems evident. Except for the pressure exerted by the short column of water over the fish in the experiments, the gas in the bladder is of the same pressure as the atmospheric air at the water surface. Under reduced pressure, tension of gases in the water favors diffusion of gases into the

bladder and atmosphere alike. The greater the decrease in pressure, the more rapid the passage of gases into the bladder.

Not up to this point explained, however, is the initial lower rate of bloating, sometimes even temporary gas output, which occurred when guppies were subjected to decreases in pressure sufficiently large to produce the bloating phenomenon. The temporary output is seen in Fig. 1 (reduction in pressure by 125 mm. Hg) and in Figs. 2*A* and 2*B* at decreases in pressure by the amount 150 (when this was carried out in two steps) and 135 mm. Hg. This temporary initial activity, an apparent attempt of the fish at adaptation, commences strongly and then gradually over the course of two to five hours becomes weaker until it is completely masked by the inward passage of gases. Either an outwardly secreting mechanism which could secrete quite efficiently up to a point of fatigue, or simple diffusion alone, might account for the response. The former explanation can neglect the partial pressures of the various gases within the swimbladder. The latter explanation is based upon the assumption that at the beginning of the experiment the bladder possesses a higher percentage of CO<sub>2</sub> than does air.

Recent work of Meesters and Nagel (1934) has shown that a fish would, under the appropriate stimulation, decrease the amount of bladder gas most rapidly when the initial percentage of CO<sub>2</sub> in the bladder was high. Thus, fish were apparently able to eliminate this gas much more rapidly than either O<sub>2</sub> or N<sub>2</sub>. The work of Jacobs (1932), confirmed by Meesters and Nagel (1934), has demonstrated that a physio-

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FIG. 3. Responses of the swimbladder to sudden decreases in pressure following immediately upon different experimental conditions of secretion and diffusion of gases to and from the bladder.

*A.* Two experiments in which guppies are subjected to a pressure decrease of 300 mm. Hg in water equilibrated with air at the reduced pressure, then returned to atmospheric pressure in water equilibrated with the restored pressure. At the moment of adaptation to the restored pressure the fishes are subjected to a sudden pressure decrease of 300 mm. Hg.

*B.* A single experiment in which a guppy was subjected to pressure increase of 260 mm. Hg and at the moment of adaptation subjected to a decrease to a point 300 mm. Hg below atmospheric pressure.

*C.* Three experiments in which guppies long (20 hours to several days) adapted to a given pressure are subjected to a sudden pressure decrease to a point 300 mm. Hg below atmospheric pressure.

*D.* Two experiments in which guppies are stimulated to decrease the gas content of their bladders and then suddenly subjected to a pressure decrease to a point 300 mm. below atmospheric pressure.

*E.* The two upper graphs indicate the response of guppies, bloating as a result of a 300 mm. Hg decrease in pressure, when they are suddenly returned to atmospheric pressure. The lower graph shows the normal response of the fish upon increase in pressure of 300 mm. Hg and then, after some hours, return to atmospheric pressure.

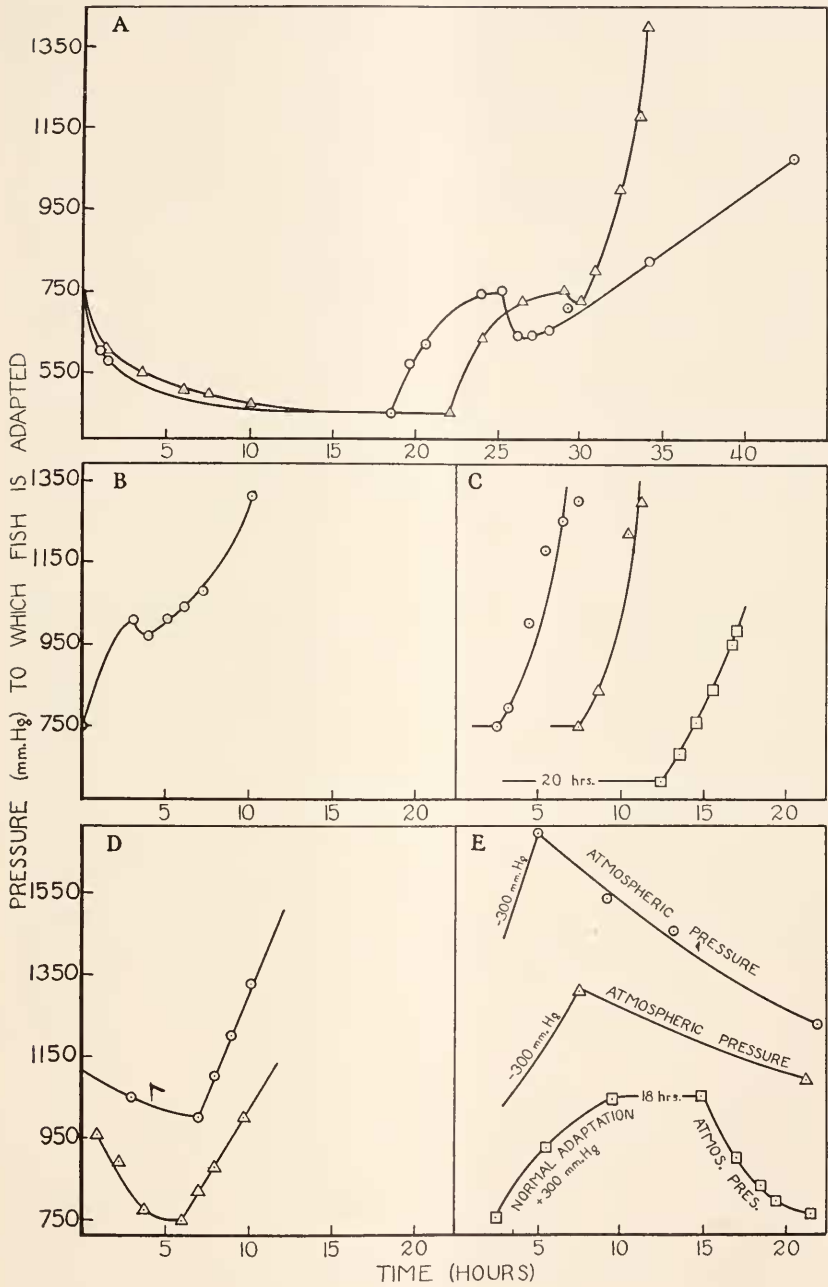


FIG. 3.



clitinous fish, the perch, secretes into its swimbladder gas which consists of about eighty per cent  $\text{CO}_2$ .

These results may be applied to a physical interpretation of the observations that have been made upon the guppy. One would merely need to assume that the fish usually possesses in its bladder a partial pressure of  $\text{CO}_2$  several times that in air at the water surface. The pressure decreases of the experiments (never more than about sixty per cent) would still favor the passage of  $\text{CO}_2$  out of the bladder while one or both of the remaining two gases would diffuse inward. The magnitude of the partial pressure of  $\text{CO}_2$  inside and the pressure decrease would determine the rate of outward diffusion of this gas and this rate might even be great enough to make the volume of the bladder decrease. Meanwhile, the response of the secretory mechanism to the pressure decrease would be one of minimal activity. As the  $\text{CO}_2$  became depleted the rate of its outward diffusion would decrease correspondingly. The inward diffusion of the gases other than  $\text{CO}_2$  would also tend to decrease the partial pressure of  $\text{CO}_2$  in the bladder. Eventually, the partial pressures of all three gases would attain the same ratio as that of atmospheric air and then all gases would diffuse inward at rates to maintain this ratio.

Indirectly, some evidence has been obtained to support the hypothesis just described. We would expect that increasing the proportion of  $\text{CO}_2$  in the bladder would permit a stronger opposition to the decrease in pressure, and a sufficient reduction in the bladder  $\text{CO}_2$  would eliminate it altogether. The following are experiments which give such support.

Two male guppies were subjected to a pressure decrease of 300 mm. Hg in water brought into gaseous equilibrium at the reduced pressure. First there was a rapid increase in fish density and then after an hour or two a considerably slower one. These two rates were interpreted on the basis of the work of Meesters and Nagel (1934) to indicate an initial rapid escape of  $\text{CO}_2$  and then the slower continued escape of other gases. The bladder was thus assumed to become relatively free of  $\text{CO}_2$  and also to contain a smaller quantity of the other gases than originally. The fish was then restored to the original pressure in water equilibrated with air at the restored pressure. The gas secreted into the bladder was assumed on the basis of Jacobs' (1932) results to be richer in  $\text{CO}_2$  than that which was withdrawn. When the proper volume of gas for the new pressure was reached and before the amount of  $\text{CO}_2$  could decrease to equilibrium with a slower secretory rate, this fish was subjected to the sudden pressure decrease of 300 mm. Hg. This fish was able to oppose much more strongly the bloating associated with this decreased pressure than was the fish of the earlier experiments. Compare the results of experiments of this nature (Fig. 3A) with the results ob-

tained when guppies had neither appreciable gain nor loss in bladder gases over some days (Fig. 3C) but subjected to the same pressure decrease.

The same general results were obtained in a slightly different manner. A guppy was stimulated to decrease its density by subjecting it to an increase in pressure of 260 mm. Hg. At the moment of adaptation to the new pressure the fish was subjected to a pressure reduction of 560 mm. Hg (Fig. 3B). This was the equivalent of the usual reduction in pressure of 300 mm. Hg.

The reverse type of experiment was next performed. Guppies, for some hours adapted to increased pressures, were stimulated to let gases escape from their bladders. On the assumption that  $\text{CO}_2$  is the gas to pass out most rapidly these fishes should, after considerable increase in density, have no larger percentage of  $\text{CO}_2$  than is present in atmospheric air. And as expected the fish were at this moment unable to oppose in the slightest the bloating effect correlated with subjection to a sudden 300 mm. Hg pressure decrease. Figure 3D illustrates results of this nature.

A final test for the hypothesis was made to ascertain that the gas present in the fish bloating rapidly under decreased pressure did not contain as large a percentage of  $\text{CO}_2$  as did the normally secreted gas. A guppy that had reached the maximum rate of bloating under the stimulus of the reduced pressure was suddenly restored to the original pressure. Under ordinary circumstances the initial response to such a decrease in pressure is a relatively rapid fish density increase which gradually or suddenly slows down. In the instance of this fish the initial response showed none of the usual initial rapidity and the whole process of return to the normal amount of gas for the pressure was relatively slow, as one would expect if the rate were determined by gases which were slow to diffuse (Fig. 3E). The normal rate of gas escape following secretion of gases into the bladder eighteen hours previously is also shown in the same figure for comparison.

#### SUMMARY

1. A sudden and maintained pressure decrease from a value of one atmosphere to points between 625 and 300 mm. Hg results in an increase in gas content of guppy swimbladders and the rate of increase is an inverse function of the pressure.
2. This increase in gas content has been shown to be the result of lowering the swimbladder gas pressure, the gas tension of the body fluids then favoring passage of gases into the bladder.

3. Guppies are able to oppose more or less successfully the inward diffusion of gases at first, but gradually in the course of two to five hours such opposition ceases.

4. The transitory attempt on the part of the fish at density adjustment in response to the pressure decreases is explainable in the presence of an initially high partial pressure of  $\text{CO}_2$  in the swimbladder.

5. The guppy appears to be unable to remove gases from the bladder when the diffusion gradient does not favor such passage.

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